

Mixed system electrochemiluminescence involving a nucleoside or a nucleotide

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Electrochemiluminescence has been generated in a mixed system through the electron transfer reaction involving riboflavin or flavinmononucleotide anion and a cation of an aromatic hydrocarbon. In each case an emission from the excited singlet state of the hydrocarbon is observed. Triplet states appear to be involved in the electron transfer reaction and it has been supported by the observation of magnetic field effect on emission intensity.

Previous reports of electrochemiluminescence (ECL) have demonstrated the formation of excited states by radical ion annihilation reactions in several mixed systems (1-6). We report here the first example of ECL of a mixed system involving a nucleotide. The ECL has been generated from reactions of riboflavin (RF) anion or flavin mononucleotide (FMN) anion with either rubrene cation or 9, 10-diphenylanthracene (DPA) cation in N, N-dimethyl formamide.

It is interesting to note that one of the molecules (FMN) studied here plays a key role in the biological electron transport chain and in bacterial bioluminescence (7-10).

The cyclic voltammetric behaviour of a solution containing RF and rubrene and 0.1 M tetra-n-butylammonium perchlorate as supporting electrolyte is shown in Figure 1. The wave I_c is identified as due to reduction of RF to riboflavin anion (RF⁻) by comparison with the cyclic voltammogram of RF (11). The reversible couple observed at wave II₁ is due to rubrene-rubrene anion (12) and that at wave I_a is due to rubrene-rubrene cation (12). The peak potentials for the different waves are listed in Table 1.

When the potential of the platinum electrode is cycled at 2.5 Hz (corresponding to the duration of forward electrolysis of $t_f = 0.2$ sec. and reverse electrolysis of $t_r = 0.2$ Sec.) between $E_{pc} = -0.60$ V and $E_{pa} = +1.30$ V, an orange—red ECL emission is observed. The emission spectrum shows a

maximum at 565nm and is shown in Figure 2. For comparison, the fluorescence spectra of riboflavin and rubrene are shown in the same figure. ECL emission is also observed when the potential swing is adjusted between wave II_c and wave I_a but the intensity of this emission is stronger by a factor of ten. (13). We could not observe an ECL emission if the potential excursion was adjusted to wave I_c and a potential short of wave I_a , or if the potential of the platinum electrode was cycled between 0 V and wave I_a (16).

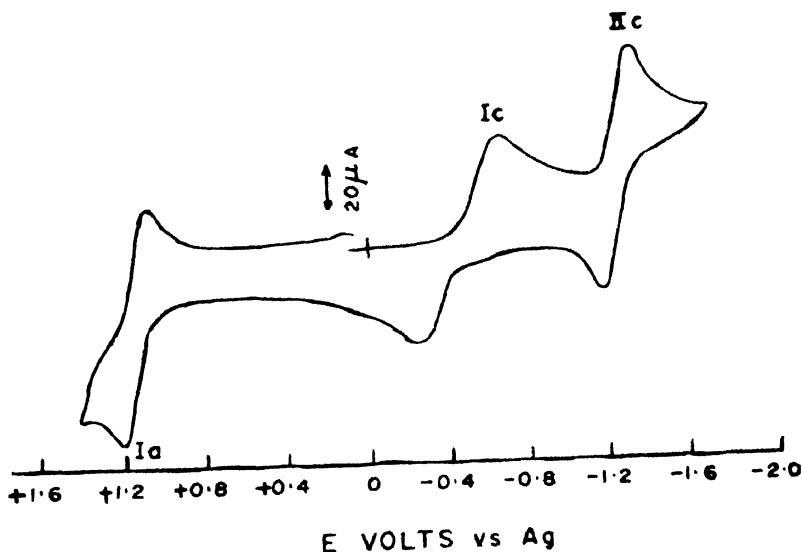
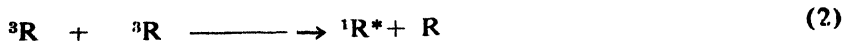


Fig. 1. Cyclic voltammogram of a solution containing 1 mM riboflavin and 1 mM rubrene and 0.1M tetra-n-butyl ammonium perchlorate recorded with a platinum working electrode at a sweep rate of 13 mv/sec

Although the fluorescence spectra of RF and rubrene overlap in certain regions of respective emissions, it is unlikely that ECL emission occurs from RF since the enthalpy (ΔH°) of the radical ion reaction is very much less than the energy of the triplet state of RF ($E_T = 2.06$ eV; $E_S = 2.84$ eV) (18). For rubrene, the spectroscopic values are $E_T = 1.2$ eV and $E_S = 2.30$ eV and the triplet state of rubrene is easily accessible in the electron transfer reaction.

On this basis the most reasonable explanation for the observation of ECL in the above experiments is the radical ion reaction between riboflavin anion and rubrene cation.



where RF = riboflavin and R = rubrene. The free radical $\text{RF}^{\cdot-}$ has previously been identified by esr (11) during electrochemical reduction at wave I_c .

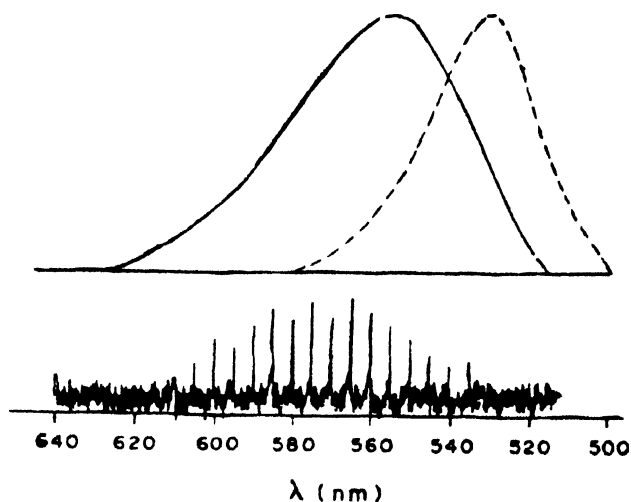


Fig. 2. Upper curve : Fluorescence Spectrum in DMF of rubrene (---) or riboflavin (----)
Lower Curve : ECL Spectrum during electrolysis of a solution containing riboflavin and rubrene obtained by cycling the potential of the working electrode between -0.60V to $+1.44\text{V}$ vs Ag.

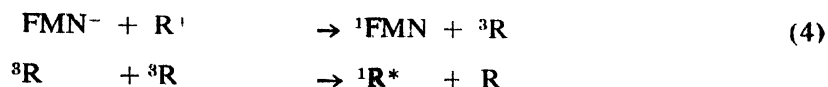
Experiments were also carried out with another oxidant DPA^+ , and the results are essentially similar. On cycling the potential of the electrode between wave I_a and wave I_c in a solution containing DPA and RF, a blue emission ($\lambda_{\text{max}} = 440\text{ nm}$) characteristic of the fluorescence of DPA is observed. This negative result is explained on the basis of the non-accessability of triplet state of RF; the values of the spectroscopic states of RF and DPA and enthalpy of the reaction ($\text{DPA}^+ + \text{RF}^{\cdot-}$) are given in Table 1.

ECL reaction involving a Nucleotide

The cyclic voltammetric pattern of a solution containing FMN and rubrene is similar to Figure 1. The peak potentials of the various waves are given in Table 1. A strong ECL emission is observed by programming the potential of the platinum electrode between wave I_c and wave I_a . The emission spectrum shows maximum at 560 nm and is attributed to emission from $^1\text{R}^*$.

Energetically, the triplet state of FMN is not accessible (see Table 1) in the electron transfer reaction.

The ECL mechanism



appears to reasonably explain the above results

Additional experiments have been conducted with a mixed system involving DPA and FMN; ECL emission is observed at 440 nm corresponding to the emission from ${}^1\text{DPA}^*$.

Table 1. Peak potentials and enthalpies^a

Mixed System	Wave I _c E _{pc} V	Wave II _c E _{pc} V	Wave I _a E _{pa} V	ΔH ^{a,b} eV
Riboflavin + Rubrene (1mM)	-0.66 (-0.82)	-1.32 (-1.48)	1.20 (1.04)	1.70
Riboflavin + 9, 10-diphenyl- anthracene (1mM)	-0.66 (-0.82)	-1.71 (-1.87)	1.51 (1.35)	2.01
Flavin Mono- nucleotide + Rubrene (1mM)	-0.68 (-0.82)	-1.32 (-1.48)	1.20 (1.04)	1.72
Flavin mono- nucleotide + 9, 10-diphenyl anthracene (1mM)	-0.68 (-0.82)	-1.71 (-1.87)	1.51 (1.35)	2.03

^aPotentials are measured with respect to Ag electrode.

Values in brackets refer to vs SEC

$E_{\text{S(RF)}} = 2.84 \text{ eV}$; $ET_{\text{T(RF)}} = 2.06 \text{ eV}$ and $E_{\text{(Rubrene)}} = 2.30 \text{ eV}$.

$E_{\text{T(Rubrene)}} = 1.20 \text{ eV}$, $E_{\text{S(FMN)}} = 2.84 \text{ eV}$; $E_{\text{I(FMN)}} = 2.05 \text{ eV}$,

$E_{\text{(DPA)}} = 3.20 \text{ eV}$ and $E_{\text{T(DPA)}} = 1.80 \text{ eV}$

(b) $-\Delta H^\circ = (E_{\text{pa}} - E_{\text{pc}}) - 0.16$

Table 2. Magnetic field enhancement of ECL intensity^a

Mag. field (kgauss)	Pulse height (volts)	% increase over initial value
0	0.28	0
1.8	0.29	3.6
2.6	0.32	14.3
3.7	0.36	28.6
4.0	0.35	28.6

^a Solution contained riboflavin and rubrene (1mM) in N, N—dimethyl formamide. Potential programming is adjusted to—0.66 V to + 1.44 V vs Ag.

The existence of triplet state intermediates in ECL mechanism is supported by the effect of a magnetic field on the emission intensities (2, 15, 17). Support for the involvement of triplets in the ECL mechanism of a mixed system involving RF or FMN is obtained from the data given in Table 2 on magnetic field enhancement of emission intensity.

The experiments conducted in this study demonstrate the intermolecular electron between FMN⁻, or RF⁻ (donor) and an acceptor (hydrocarbon cation) in producing the triplet states. The sugar and/or phosphate present in the donors together with an associated solvation do not appear to localise the odd electron which would slow down the electron transfer reaction and thus render difficult the detection of the ECL emission.

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